HAEMATOLOGICAL PROFILE IN THE DIFEERENTIAL DIAGNOSIS OF MICROCYTIC HYPOCHROMIC ANAEMIA IN CHILDREN

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CERTIFICATE

This is to certify that the dissertation entitled **Haematological profile in the differential diagnosis of Microcytic Hypochromic Anaemia in Children -** is a bona fide work done by **Dr.Karthika.V,** postgraduate student in the department of pathology, Coimbatore Medical College, Coimbatore under the supervision of **Dr.M.Murthy,MD,** Professor & Head, Department of Pathology, Coimbatore Medical College, Coimbatore and under the guidance of **Dr.C.Lalitha,MD,** Additional Professor, Department of Pathology, Coimbatore Medical College, Coimbatore in partial fulfillment of requirements of the Tamilnadu Dr. MGR Medical University for the award of MD Degree in Pathology.

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INTRODUCTION:

Anaemia, whether clinically overt or not is a common condition encountered by the family physician. The most commonly encountered disorders manifesting with mild microcytic hypochromic anaemia are iron deficiency anemia (IDA) and Thalassemia Trait (TT)[1,2]. Hypochromic microcytic aneamia could also be due to anaemia of chronic disease or lead poisoning. The establishment of an accurate diagnosis is of great importance in ensuring correct treatment. Administration of iron to a patient with hypochromic aneamia due to a cause other than iron deficiency is not only useless but also leads to undesirable effects of increase in body iron stores. Thalassemia Minor and Thalassemia Intermedia may pass off as iron deficiency anaemia, if only peripheral smear was the sole diagnostic modality.

β-Thalassemia is also an iron loading anemia, meaning that thalassemic patients have a dramatic increase in iron absorption from the gut due to their increased erythropoietic rate[3–6]. Together with the iron influx from chronic transfusions the setting of iron overload is observed in thalassemic patients. Inadvertent iron therapy will prove detrimental in such situations.

Iron plays an essential role in many important biochemical processes [7]. As with all nutrients, the requirement for iron is greater during periods of rapid growth and differentiation such as in the late fetal and neonatal period.

Consequently, poor iron homeostasis during this period can result in disordered development. Inadequate tissue iron levels can lead to reduced erythropoiesis and poor O_2-carrying capacity. The nervous system, which develops rapidly during the late fetal and early neonatal period, seems to be particularly susceptible to iron deficiency and excess [8]. Also, Iron excess can have severe effects on neuron development [6–8]. Thus, events occurring in early life can have long-lasting effects on neuronal function in the adult. Excessive iron in the circulation leads to abnormal accumulation in organs such as liver, spleen and heart, leading ultimately to liver disease, cardiac dysfunction, arthropathy, gonadal insufficiency and other endocrine disorders (**Hoffman et al., Hematology, Basic Principles and Practice**[15]).

Twenty percent of children in US and eighty percent of children in developing countries are anaemic at some point of time by the age of 18 years (**American fam physician 2001:64:1364-79**). Hence it is worthwhile to study the hematological profile including serum iron, serum iron binding capacity, haemoglobin electrophoresis and bone marrow iron which would delineate these disease entities and enable correct management. Timely management of anaemia in growing buds is very important especially for a developing country like India as the children of today are the best national resource of tomorrow.

AIM OF THE STUDY

1. To find out causes of microcytic hypochromic anaemia in children & their respective prevalence.
2. To study red cell indices in various types of hypochromic microcytic anaemia.
3. To analyze the Serum Iron, Total Iron Binding Capacity, Serum Ferritin levels in various types of hypochromic microcytic anaemia.

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REVIEW OF LITERATURE

DEFINITION:

Anaemia is defined as a reduction in the concentration of circulating haemoglobin or oxygen carrying capacity of blood below the level that is expected for healthy persons of same age and sex in the same environment.

PREVALENCE:

Anaemia remains a widespread public health problem with major consequences for human health as well as social and economic development. Although estimates of the prevalence of anaemia vary widely and accurate data are often lacking, it can be assumed that significant proportions of young children and women of childbearing age are anaemic (**WHO** 2001, 2003). Preschool children are more affected than women, with reported prevalence in excess of 60% in many countries. Nutritional deficiency constitutes most common cause of anaemia (Iron, B12, & Folic acid).

Genetic disorders, particularly Thalassaemia, remain another possibility. In a multicity study, the incidence of Thalassemia Trait has been found to be 2.7% in Bombay, 5.5% in Delhi & 10.4% in Calcutta (ICMR). It is estimated that about 10,000 children with Thalassemia Major are born every year in India, accounting for 10% of Thalassemia Major birth worldwide.

Thalassemia primarily affects people of Mediterranean, Southeast Asian, and African ancestry in malaria-endemic regions [**J Nippon med Sch 2004;71**[12]].

THE PREVALENCE AND DISTRIBUTION OF IRON DEFICIENCY WORLDWIDE:

Iron deficiency is the most prevalent and the most common micronutrient deficiency in the developing world today (**Tatala et al**,1998[16]; **Asobayire et al.**2001[17];**Abalkhail and Shawky**, 2002[18];**Hashizume et al**.2003[19]).

The prevalence of iron deficiency varies widely depending on the criteria used to establish the diagnosis. Variables include age, socioeconomic status, family size, nutritional status and total income of the family. According to UNICEF report, two billion people suffer from anemia worldwide and most of them have iron deficiency anemia, especially in underdeveloped and developing countries, where 40-50% of children are iron deficient (**UNICEF**). According to world health organization (**WHO**), there are no current global figures for iron deficiency anemia, but using anemia as an indirect indicator 39-48% children in non industrialized countries compared to 6-20% in industrialized countries are iron deficient as shown in table (**WHO, 2001**).

Data presented in shows regions with the numbers of anemic cases in these regions as reported by **WHO** (WHO, 2001).

Percentage of affected population		
Age group/y	Industrialized Countries	Non-industrialized Countries
0-4 years	20.1	39
5-14 years	5.9	48.1
Females 15-59 years	10.3	42.3
Males 15-59 years	4.3	30

HEMATOPOIESIS

EMBRYONIC HEMATOPOIESIS

Hematopoiesis and vasculogenesis in the mammalian embryo begin in the blood islands of the yolk sac and continue, somewhat later, within the embryo proper. Blood islands are formed from mesodermal aggregates that have migrated from the primitive streak. The outer cells differentiate into endothelial cells and the inner to primitive blood cells. The close developmental association between hematopoietic and endothelial cell lineages has led to a hypothesis that they share a common progenitor, the hemangioblast. The mechanisms that control formation of hemangioblast and embryonic hematopoietic and endothelial (angioblastic) stem/progenitor cells are still not well understood (**oxford textbook**).

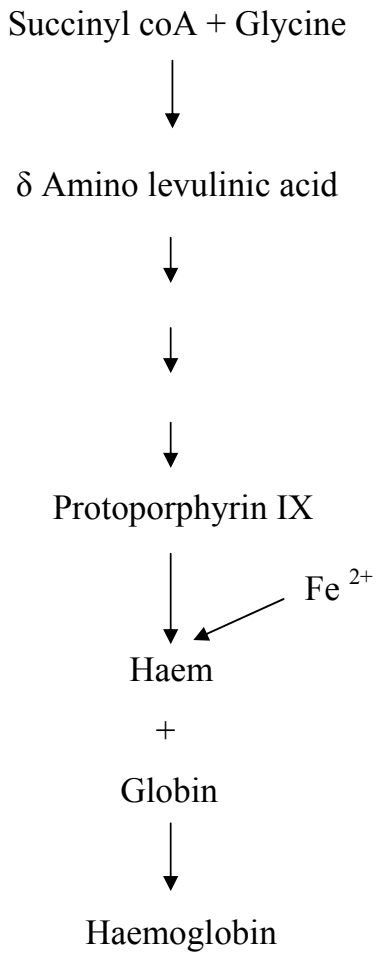
Erythropoiesis takes place within the primitive circulation (intravascular) and is megaloblastic in type. Multipotent progenitors derived from the mesoderm are present at early stages of gestation. For many years, investigators have believed that yolk sac blood islands contained Hematopoietic Stem Cells (HSC) capable of primitive hematopoiesis and of migration to the developing liver to initiate definitive hematopoiesis. Challenging the idea of a singular origin of hematopoiesis in the yolk sac, it has been proposed that there is a more potent intraembryonic HSC site in the Aorta-Gonado-Mesonephros (AGM) region. HSCs arise for the first time in the AGM region and migrate to the yolk sac and fetal liver, the main source of hematopoietic cells in fetal life. Around the time of birth, HSCs migrate from the liver to the bone marrow, to be responsible for adult hematopoiesis.

At about sixth week of gestation, erythropoiesis begins in the fetal liver extravascularly, with mature cells entering the fetal circulation. Erythropoiesis is also detectable in the spleen by the 12^{th} week, this remaining the primary site of erythropoiesis untilthe 24^{th} week.

BONE MARROW HAEMATOPOIESIS:

This commences at around week 16 to 18, as fetal liver hematopoiesis is challenged by hepatocyte proliferation, and assumes the primary role from week 24 onward.

Haem is complex of protoporphyrin and iron.



GLOBIN SWITCHING:

An orderly sequence of production of different globin proteins occurs during fetal development in response to changes in requirements for red blood cell oxygen-carrying capacity. The earliest globin chains detectable in the embryo yolk sac are zeta, Alpha type chain with locus on chromosome 16, and epsilon (ε), a β-type chain with locus on chromosome 11. The earliest fetal haemoglobin is thus HbGower1, and it is the major form at 5 to 6 weeks.

HbGower 2 is present from 4 to 13 weeks of gestation. HbPortland also persists from 4 to 13 weeks but is found in infants with homozygous Thalassemia. HbF is the major fetal form and accounts for 90 to 95% of the total haemoglobin until 34 to 36 weeks gestation. Adult haemoglobin (HbA) is detectable from week 11 of gestation, after which time the proportion of HbA increases as HbF declines. The amount of HbF in neonates varies from 50 to 90%, but thereafter declines at a rate of 3% per week and is generally less than 2 to 3% by the age of 6 months.

Relative proportions & constitutions of different haemoglobins are;

Adults:

Hb A - $\alpha_2\beta_2$ - 97%

Hb A2 - $\alpha_2\delta_2$ - 2.5%

Hb F - $\alpha_2\gamma_2$ - 0.5

Newborn:

Hb F - $\alpha_2\gamma_2$ - 80%

Hb A - $\alpha_2\beta_2$ - 20%

Embryonic Hb:

Hb Gower I - $\zeta_2\varepsilon_2$

Hb Gower II- $\alpha_2\varepsilon_2$

Hb Portland - $\varepsilon_2\gamma_2$

FETAL BLOOD CELL VALUES

Haemoglobin concentration rises from a mean of 11.7 g/dl at 18 weeks to 13.6 g/dl at >30 weeks, with a steady rise in hematocrit (0.37 l/l to 0.43 l/l) and concomitant fall in mean cell volume (131 fl to 114 fl). Circulating normoblasts constitute 45% of nucleated cells at 18 weeks, falling to 17% at >30 weeks. Lymphocyte percentage falls from 88% to 68%, with neutrophils only rising significantly after 30 weeks (8% at 26 to 29 weeks to 23% at >30 weeks). Eosinophil, monocyte, and basophil percentages remain reasonably constant throughout. Platelet concentration also remains constant. (**Oxford Handbook**).

MEASUREMENT OF ERYTHROPOIESIS

Normal red blood cell production is extremely effective, and most red blood cells live, or have the potential to live, a normal life span. Under certain conditions, however, a fraction of red blood cell production is ineffective, with destruction of nonviable red blood cells either within the marrow or shortly after the cells reaches the blood.

EFFECTIVE ERYTHROPOIESIS

It is most simply estimated by determining the reticulocyte count. This count is usually expressed as the percentage of red blood cells that are reticulocytes, but it can also be expressed as the total number of circulating reticulocytes per liter of blood (reticulocyte % × RBC per l). An elevated

reticulocyte count may give an erroneous impression of the actual rate of red cell production because of premature release of reticulocytes into the circulation.

INEFFECTIVE RED BLOOD CELL PRODUCTION:

Ineffective erythropoiesis is suspected when the reticulocyte count is low or is normal or only slightly increased in the presence of erythroid hyperplasia in the bone marrow. In certain disorders, such as Addisonian pernicious anemia, Thalassemia, and sideroblastic anemia, ineffective erythropoiesis is a major component of total erythropoiesis. This can be quantified by ferrokinetics. Using ferrokinetic methods, ineffective erythropoiesis is calculated as the difference between total plasma iron turnover and erythrocyte iron turnover plus storage iron turnover.

HEMATOPOIETIC REGULATION

The maintenance of hematopoiesis in steady state by a balance of negative and positive cytokine regulators. A variety of the cytokines that include the Hematopoietic Growth Factors (HGFs), Stem Cell Factor (SCF), flt3 ligand (FL), Thrombopoietin (TPO), Interleukin-3 (IL-3), Granulocyte/ Macrophage Colony Stimulating Factor (GMCSF), and IL-6 have been shown, in various combinations, to promote the growth and differentiation of

Hematopoietic Stem Cells (HSCs). HSC proliferation is intimately linked to the stromal cells and Extra Cellular Matrix (ECM) in distinct microenvironmental niches. They composed of a variety of molecules, including fibronectin, laminin, collagens, and proteoglycans. Some components of the ECM bind to cytokines produced by the stroma, immobilizing them within the microenvironmental niches and thus creating a zone in which HSCs and cytokines can coalesce.

IRON METABOLISM

Most of the iron is present in the oxygen carrying protein of the red blood cells-haemoglobin. Iron turnover is also dominated by the synthesis and breakdown of haemoglobin. Haem is synthesized in nucleated red cells in the bone marrow by a pathway ending with the incorporation of iron into protoporphyrin IX by ferrochelatase. Haem breakdown takes place in phagocytic cells, largely those in the spleen, liver and bone marrow. Iron is released from haem by haem oxygenase and is largely reused for haem synthesis. Every day about 30 mg of iron are used to make new haemoglobin and most of this obtained from the breakdown of old red cells.

Relatively little iron is lost from the body (about 1mg/day in men) and these losses are not influenced by body iron content or the requirement of the body iron. The body iron content is maintained by variation in amount of iron absorbed. In most men and postmenopausal women there is some storage

iron. This is iron in ferritin or its insoluble derivative haemosiderin which is available for haem synthesis if necessary.

DIETARY IRON ABSORPTION

Iron absorbed depends on [13]

- The amount of iron in the diet
- Its bioavailability
- The body's need for iron
- Age and
- Inflammatory states with hepcidin release

Nonhaem iron is released from food as Fe 3+ and reduced to Fe2+ by a membrane – bound ferrireductase, Dcytb.

Iron is transported across brush-border membrane by the metal transporter, DMT-1. Some iron is incorporated into ferritin and lost when the cells are exfoliated.

Iron destined for retention by the body transported across the serosal membrane by ferroportin-1. Before uptake by transferrin, Fe2+ is oxidised to Fe3+ by hephaestin or by plasma ceruloplasmin.

Haemoglobin and myoglobin are digested in the stomach and small intestine. Haem is initially bound by haem receptors at the brush border membrane and the iron is released intracellularly by haem oxygenase before

entering the labile iron pool and following a common pathway with iron of nonhaem origin[14].

REGULATION OF IRON ABSORPTION

Iron absorption may be regulated both at the stage of mucosal uptake and at the stage of transfer to the blood. As epithelial cells develop in the crypts of Lieberkuhn their iron status reflects that of plasma (transferrin saturation) and this programmes the cells to absorb iron appropriately as they differentiate along the villus.

Transfer to the plasma depends on the requirements of the erythron for iron and the level of iron stores. This regulation is mediated directly by hepcidin, a peptide synthesized in the liver in response to iron and inflammation. Hepcidin blocks intestinal iron absorption and iron release from liver and spleen. The main mechanism by which hepcidin exerts its effects appears to be control of ferroportin. Indeed, ferroportin is the only known cellular iron exporter which cannot mediate iron release from the cell once hepcidin exerts its actions.

CELLULAR IRON UPTAKE AND RELEASE

Transferrin binds to the transferrin receptor (TfR) lining the cell. The two proteins bind strongly to form a high affinity complex which

initiates endocytosis of the local membrane. The resulting endosome contains the transferrin-transferrin receptor complex. The pH of the endosome is then reduced by a proton pump to induce a conformational change in holotransferrin, which releases its iron. Iron is transported into the cell via DMT-1. This iron is then either stored as ferritin or used within the cell (for Hb synthesis in erythroid precursors). The apotransferrin and transferrin receptor return to the cell surface where they dissociate at neutral pH so that the cycle can start again.

The reticuloendothelial macrophages play a major role in recycling iron resulting from the degradation of haemoglobin from senescent erythrocytes. They engulf red blood cells and release the iron within using haem oxygenase. The iron is rapidly released to plasma transferrin or stored as ferritin. Little is known about the mechanism of release, but ferroportin 1 may be as essential component.

Ferritin is found in all the cells and in the highest concentration in liver, spleen and bore marrow[14].

With the recognition that the small quantity of ferritin in human serum (15-300 μg/L in healthy men) reflects body iron stores, measurement of serum ferritin has been widely adopted as a test for iron deficiency and iron overload.

IRON NEEDS DURING INFANCY AND CHILDHOOD

To meet the needs of iron for growth and to replace normal losses, iron intake must supplement the approximately 75 mg of iron per kilogram of body weight that is present at birth (**Widdowson, Spray, et al.**[21]). Iron losses from the body are small and relatively constant. About two thirds of iron losses in infancy occur when cells are extruded from the intestinal mucosa and the remainder when cells are shed from the skin and urinary tract. In the normal infant, these losses average approximately 20 mg per kilogram per day. An infant who weighs 3kg at birth and 10kg at one year of age will require approximately 270 to 280mg of additional iron during the first year of life to maintain normal iron stores [**Widdowson et al.**[21]].

Breast milk and cow's milk both contain about 0.5 to 1.0mg of iron per liter, but its bioavailability differs markedly. The absorption of iron from breast milk is uniquely high, about 50 percent on an average and tends to compensate for its low concentration (Bioavailability). In contrast, only about 10% of the iron in whole cow's milk is absorbed. About 4% of iron is absorbed from iron fortified cow's-milk formulas that contain 12mg of iron per liter (**Saarinene et al.**[22]**, McMillan et al**.). The reasons for the high bioavailability of iron in breast milk are unknown, although it appears that the high concentrations of calcium, phosphorus, and protein, in conjunction with the low concentration of ascorbic acid, are responsible, in part, for the poor absorption of iron from cow's milk.

After one year of age, the diet becomes more varied and there is less information from studies on which to base dietary recommendations. The recommended dietary allowance decreases to 10mg per day for children between 4 and 10 years of age and then increases to 18mg per day at the age of 11 to provide for the accelerated growth that take place during adolescence (**Elk et al.**[23]). Two thirds of body iron is present in circulating red blood cells as haemoglobin. Each gram of haemoglobin contains 3.47 mg of iron; thus, each ml of blood lost from the body (haemoglobin 15 g/dl) results in a loss of 0.5 mg of iron (**Conrad et al.**).

Hookworms also reported as other causative bleeding agents as 35% of ID total reported to suffer from hookworm infections. Necator americanus or Ancylostoma duodenale are the most common parasitic species involved in bleeding; however, further investigation is required for the identification of such parasites (**Hopkins et al.**[25]).

IRON DIFICIENCY ANAEMIA

Iron balance is usually achieved by regulation of iron absorption in the proximal small intestine. Either diminished absorbable dietary iron or excessive loss of body iron can cause iron deficiency.

Diminished absorption is usually due to an insufficient intake of dietary iron in the absorbable form. While body loss of iron quantitatively is

as important as absorption in terms of maintaining iron equilibrium, it is a more passive process than absorption. Consistent errors in maintaining this equilibrium lead to either iron deficiency or iron overload (**Conrad et al.**). Also some constituents present in the food regulate the absorption of Iron. Ascorbic acid enhances the absorption of non-heme iron, as do meat, fish, and poultry (**Derman et al.**[26]). Inhibitors of absorption include bran, polyphenols, oxalates, phytates, vegetable fiber, the tannins in tea, and phosphates (**Charlton and Bothwell et al.**[27]).

Iron-deficiency anemia can be the consequence of several factors, including:

- Insufficient iron in the diet
- Poor absorption of iron by the body
- Ongoing blood loss, most commonly from menstruation or from gradual blood loss in the intestinal tract
- Periods of rapid growth
- Damage of intestines
- Infection and disturbance of mucosa
- Elevation of pancreatic secretions

Children between 1 and 3 years of age are at risk of iron deficiency and iron-deficiency anemia, even though it is not a period of exceptional growth. Most toddlers are no longer consuming iron-fortified formula and infant cereal, and they are not eating enough iron-rich foods to make up for the

difference. Toddlers also tend to drink a lot of cow's milk, often more than 24 ounces a day. During the first stages of puberty, when growth spurt occurs, boys are at risk of iron-deficiency anemia. Adolescent girls are at higher risk because of menstrual blood loss and smaller iron stores when compared to boys (**Christopher et al.**[28]).

SYMPTOMS OF IRON DEFICIENCY ANEMIA

Many people with iron deficiency anemia will not suffer from additional symptoms; however several common symptoms of iron deficiency anemia are well defined.

The symptoms include:

- Headache,
- Abnormal pallor or lack of color of the skin,
- Irritability,
- Lack of energy or tiring easily (fatigue),
- Increased heart rate (tachycardia),
- Sore or swollen tongue,
- Enlarged spleen,
- A desire to eat peculiar substances such as dirt or ice in large amounts.

DIAGNOSIS OF IRON DEFICIENCY ANEMIA

Iron-deficiency anemia develops as an end result of a series of steps that begin with depletion of stored iron. First, iron disappears from the bone marrow, and the red-cell distribution width becomes abnormal. Next, there is a loss of transport iron, reflected by a reduced serum iron level. Then, erythropoiesis becomes iron-deficient, as indicated by a reduced mean corpuscular volume and an increased concentration of red-cell protoporphyrin. The result is overt anemia.

Diagnosis of moderate or severe iron-deficiency anemia is easy. The disease is characterized by low MCV, reduced serum ferritin level, reduced serum iron level, increased serum iron-binding capacity, increased red-cell protoporphyrin level, and increased red-cell distribution width. The diagnosis of mild forms of iron-deficiency anemia may present a greater challenge. The laboratory tests may be less reliable, and the values of iron deficient and iron-sufficient persons overlap considerably (**Charlton et al., Yip et al.**[27]). The following represent general considerations:

- A complete blood count (CBC) may reveal low haemoglobin levels and low hematocrit (the percentage of red blood cells in whole blood).
- The CBC also gives information about the size of the red blood cells (RBCs). RBCs with low haemoglobin tend to be smaller and less pigmented (Microcytic and hypochromic).

- Serum iron directly measures the amount of iron in blood, but may not accurately reflect iron concentrations in cells.
- Serum ferritin reflects total body iron stores. It is one of the earliest indicators of depleted iron levels, especially when used in conjunction with other tests, such as (CBC).

The most useful single laboratory value for the diagnosis of iron deficiency may be plasma ferritin. Ferritin is the cellular storage protein for iron. Plasma ferritin differs from its cellular counterpart in several respects, and appears to be a secreted protein of different origin (**Arosio et al.**[29]). Plasma ferritin values often falls under 10% of its baseline levels with significant iron deficiency.

ANAEMIA OF CHRONIC DISEASE

PATHOPHYSIOLOGY

Anaemia of chronic disease is of immunological origin. In clinical setting, it is mostly found in the patients with chronic inflammatory process, chronic infectious disease and in patients with malignant tumors. Perturbations in iron homeostasis can be found that are associated with an increased uptake and retention of iron in the cells of the reticuloendothelial system. This leads to the deposition of iron in the cells of the reticuloendothelial system, which, in turn yields a lack of iron for

erythropoiesis. The increase in iron storage appears to be mediated by pro-inflammatory cytokines.

Tumor necrosis factor-alpha, for example, inhibits synthesis of the iron storage protein ferritin in macrophages and hepatocytes. Additionally, the expression of the membrane protein DMT1 is upregulated by interferon-gamma, bacterial lipopolysaccharide and tumor necrosis factor-alpha. This protein mediates iron transport into the intestinal mucosal cell as well as into activated macrophages. Moreover, export of iron from these cells is inhibited through down regulation of the expression of ferroportin.

Hepcidin appears to play a central role in this setting, because it is more abundantly expressed under the influence of lipopolysaccharide and interleukin-6 secretion. This, in turn, yields an additional inhibition of iron absorption from the gut. Furthermore, the action of aforementioned cytokines, especially that of interferon-gamma, leads to a direct suppression of erythropoiesis. It has been proposed that the responsible mechanisms are an induction of apoptosis and inhibition of erythropoietin expression.

In addition to the effects of iron sequestration, inflammatory cytokines promote the production of white blood cells. Bone marrow produces both red blood cells and white blood cells from the same precursor stem cells. Therefore, the upregulation of white blood cells causes fewer stem cells to

differentiate into red blood cells. This effect may be an important additional cause for the decreased erythropoiesis seen in anaemia of inflammation, even when erythropoietin levels are normal, and even aside from the effects of hepcidin.

In the short term, the overall effect of these changes is likely positive: it allows the body to keep more iron away from bacterial pathogens in the body, while producing more immune cells to fight of infection. Bacteria, like most living forms, depend on iron to live and multiply. However, if inflammation continues, the effect of locking up iron stores is to reduce the ability of the bone marrow to produce red blood cells.

CLINICAL PROFILE

The anaemia of chronic disease presents itself as a normochromic normocytic anaemia[14]. It normally does not lead to a decrease in haemoglobin below 8 g/dl. The diagnosis is usually established by a low serum iron concentration, a low transferrin concentration, a low transferrin saturation, and normal or increased ferritin values in the presence of chronic illness[30,31]. The number of reticulocytes is usually low, which points to a small rate in de novo production. Serum values of iron and transferrin saturation are normally reduced, because the iron is trapped inside the rediculoendothelial system[32]. Assessing C-reactive protein values is usually helpful to differentiate acute inflammatory process[31].

β-THALASSEMIA

PATHOPHYSIOLOGY OF β-THALASSEMIA:

Thalassaemia is inherited as an autosomal recessive disorder characterized by a microcytic hypochromic anaemia, and a clinical phenotype varying from almost asymptomatic to a lethal haemolytic anaemia. It is characterized by decreased or absent globin chain synthesis and by ineffective erythropoiesis. It is classified according to the defective globin chain and clinical severity.

Individuals who are homozygotes for β-Thalassemia genes (β+/β+, β0/β0) have a severe, transfusion dependent anaemia called β-Thalassemia Major. Heterozygotes with one Thalassemia gene and one normal gene (β+/β or β0/β) usually have a mild microcytic anaemia that causes no symptoms. This condition is known as Thalassemia Minor or Thalassemia Trait. Thalassemia Intermedia are is the third genetic variant of the β-Thalassemias,(β+/β+ or β0/ β) which incorporates a less severe anemia than Thalassemia Major with inefficient erythropoiesis as well as peripheral hemolysis.

Three main sequelae which are responsible for the clinical manifestation of Thalassemia Intermedia are ineffective erythropoiesis, chronic anemia, and iron overload. Ineffective erythropoiesis is due to imbalance between the β, α chain synthesis. Free α chains precipitate within the normoblast, forming insoluble inclusions. These inclusions cause cell

membrane damage, and premature destruction within the bone marrow leading to ineffective erythropoiesis, and also lysis of premature red cells in the spleen (hemolysis).

Leg ulcers, pulmonary arterial hypertension, extramedullary hematopoiesis, and thrombotic events are some of the complications that Thalassemia Intermedia patients. Thalassemia Intermedia encompasses a wide clinical spectrum. Mildly affected patients are completely asymptomatic until adult life, experiencing only mild anemia and maintaining haemoglobin levels between 7 to 10 g/dl. These patients require only occasional blood transfusions, if any. Patients with more severe Thalassemia Intermedia generally present between the ages of 2 and 6 years, and although survival does not depend on regular transfusion therapy, growth and development can be retarded. Thalassemia Minor is more common than Thalassemia Major (**Robbins**). These patients are usually asymptomatic; anaemia is mild.

Recognition of β Thalassemia Trait is important on two accounts:

1. Differentiation of microcytic hypochromic anaemia from Iron deficiency anaemia.
2. Genetic counseling.

Even though prevalence of anaemia is less common in Japan, a study conducted in junior high school children, two children with hypochromic microcytic anaemia were found to be diagnosed as Thalassemia Intermedia.

[J Niipon Med Sch 2004: 71[12]]. This shows the importance of screening for hypochromic microcytic anaemia.

Differentiation can be reasonably made between Iron deficiency anaemia and β Thalassemia Trait using Red cell counts. Elevated Red cell count especially in the presence of mild anaemia was a reliable indicator of β Thalassemia Trait.(**Nishi madhan et al**.) in this study 287 Trait had an elevated RBC count in contrast to 9 in Iron deficient subjects.

DIAGNOSIS

CLINICAL DIAGNOSIS

Thalassemia Major is usually suspected in an infant younger than two years of age with severe microcytic anemia, mild jaundice and hepatosplenomegaly. Thalassemia Intermedia presents at a later age with similar but milder clinical findings. Carriers are usually asymptomatic, but sometimes may have mild anemia. Hematologic Diagnosis **RBC indices** show microcytic anemia. Thalassemia Major is characterized by reduced Hb level (<7 g/dl), mean corpuscular volume (MCV) > 50 < 70 fl and mean corpuscular Hb (MCH) > 12< 20 pg.

Thalassemia Intermedia is characterized by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Thalassemia Minor is characterized by reduced MCV and MCH, with increased Hb A2 level[35].

PERIPHERAL BLOOD SMEAR

- Affected individuals show RBC morphologic changes [microcytosis, hypochromia, anisocytosis, poikilocytosis (speculated tear-drop and elongated cells), and nucleated RBC (i.e., erythroblasts). The number of erythroblasts is related to the degree of anemia and is markedly increased after splenectomy.
- Carriers have less severe RBC morphologic changes than affected individuals. Erythroblasts are normally not seen. Qualitative and quantitative Hb analysis (by cellulose acetate electrophoresis and DE-52 microchromatography or HPLC) identifies the amount and type of Hb present. The Hb pattern in β-Thalassemia varies according to β-Thalassemia type. In beta0 Thalassemia, homozygotes HbA is absent and HbF constitutes the 92-95% of the total Hb. In beta+ Thalassemia homozygotes and beta+/beta0 genetic compounds HbA levels are between 10 and 30% and HbF between 70-90%. HbA2 is variable in β-Thalassemia homozygotes and it is enhanced in beta Thalassemia Minor.

MOLECULAR GENETIC ANALYSIS

- The prevalence of a limited number of mutations in each population has greatly facilitated molecular genetic testing. Commonly occurring mutations of the beta globin gene are detected by PCR-based

procedures [36]. The most commonly used methods are reverse dot blot analysis or primer-specific amplification, with a set of probes or primers complementary to the most common mutations in the population from which the affected individual originated.

- If targeted mutation analysis fails to detect the mutation, beta globin gene sequence analysis can be used to detect mutations in the beta globin gene.

Table-4; Serum values in various hematological profiles of microcytic hypochromic anaemia:

Parameter	Iron deficiency Anaemia	β-Thalassemia	Anaemia of chronic Desease
Iron	↓	↑	↓
Transferrin Saturation	↓	↑	↑
Ferritin	↓	↑	↑
S. transferrin Receptor	↑	↔	Normal
Ratio of S. transferrin receptor to log ferritin	High (>2)	Low (<1)	↓
Cytokines level	↔	↑	↑

RED CELL INICES AND FUNCTIONS:

Several studies have derived discriminatory functions based on RBC indices that can be used to differentiate between patients with Thalassemia and those with Iron deficiency anaemia (**Shine and lal et al**.[39]).

Red cell indices used to are;

- Mentzer index (MI)
- Shine and lal index (S&L)
- England and Fraser index (E &F)
- Srivastava index (S)
- Green and King index (G&K)
- RDW index (RDWI)
- Ricerca index (R)

A study conducted by **V Okan, A Cigilogu, S cifci et al**[47], calculated the sensitivity, specificity, Positive predictive value, Negative predictive value of these nine indices. The study included 100 β Thalassemia Trait cases and 100 Iron deficiency anaemia in the age group range of 15- 87 years. They found that Shine and Lal and Green & King indices to be the best at differentiating Iron deficiency anaemia from Thalassemia patients and RDW index to be the worst.

These indices have been proposed during the past 35 years to discriminate between the two (**Sirdah et al.**[42]). An ideal discrimination index should have high sensitivity and specificity and is easy to calculate. (M.A. **Ehsani et al.**[53])

PERIPHERAL SMEAR EXAMINATION:

MICROCYTES:

Microcytes usually result from defect in haemoglobin synthesis. It should be distinguished from red cell fragmentation. Both abnormalities can lead to a reduction in the mean cell volume. However low MCV is common in association with a defect in Haemoglobin synthesis, where as it is uncommon in fragmentation syndromes because the fragments usually comprise only a small percentage of erythrocytes.

HYPOCHROMASIA:

Normal central pallor will be increased due to defective haemoglobin synthesis.

BASOPHILIC STIPPLING:

It denotes the presence of numerous basophilic granules distributed throughout the cell. Basophilic stippling is indicative of disturbed rather than increased erythropoiesis. It occurs in conditions other than Thalassemia, which includes megaloblastic anaemia, infections, liver disease and lead

poisoning (**Dacie and Lewis**). Coarse basophilic stippling is defined as easily identified, uniformly distributed basophilic inclusions.

PREKERATOCYTES:

They are RBCs with 1 or more sharp-edged, submembranous vacuoles and central pallor. They are commonly observed in Iron deficiency anaemia **(Alexandara et al).**

PENCIL CELLS:

They are elongated, hypochromic RBCs, in which the long axis was more than 3 times the length of the short axis, more commonly seen in IDA compared to Beta TT.

TARGET CELLS:

They are RBCs with a central haemoglobinized area surrounded by an area of pallor. They are more commonly observed in Beta TT compared to IDA.

MATERIAL AND METHODS

STUDY DESIGN:-

Prospective study

STUDY POPULATION:-

Patients aged 1-12 years with microcytic hypochromic anaemia.

SAMPLE SIZE:-

A total of forty patients with hypochromic microcytic anaemia were subjected to assessment of hematological profile. Approximately 1200 samples of hypochromic microcytic anaemia are received in the department of pathology per year representing 3.67% of incidence.

STUDY PERIOD:-

One and a half years [February 2009 to August 2010].

METHODOLOGY:-

Patients aged 1-12 years with microcytic hypochromic anaemia are selected with following inclusion and exclusion criteria.

INCLUSION CRITERIA:-

- Age group 1-12 years.
- Patients with clinical symptoms of anaemia.
- Haemoglobin level: Age 1-6 years <10.5 g/dl, 7-12 years <11 g/dl.

II. EXCLUSION CRITERIA:-

- Age below one year and above twelve years.
- Peripheral smear with dimorphic picture.
- H/O transfusion within past two months.
- Children on haematinics.

REFERENCE VALUE (dacie and lewis)**:**

Indices	1 year	2- 6 years	6- 12 years
Red cell count $x10^{12}$/l	4.5(+/-)0.6	4.6 (+/-)0.6	4.6(+/-)0.6
Haemoglobin g/l	126(+/-)15	125(+/-)15	135(+/-)20
PCV l/l	0.34(+/-)0.04	0.37(+/-)0.03	0.40(+/-)0.05
MCV fl	78(+/-)6	81(+/-)6	86(+/-)9
MCH pg	27(+/-)2	27(+/-)3	29(+/-)4
MCHC g/l	340(+/-)20	340(+/-)30	340(+/-)30
Reticulocyte $x10^{9}$/l	30-100	30-100	30-100

[PCV- Packed Cell Volume, MCV- Mean Cell Volume, MCH- Mean Cell Hb, MCHC – Mean Cell Hb Concentration].

Complete hemogram was done using sysmex KX-21, semi automated haematology analyzer (Manufacturer: Sysmex corporation, Japan).

DETECTION PRINCIPLE:

This instrument performs blood cell count by DC detection method. Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into the each transducer. The transducer chamber has a minute hole called aperture. On both sides of the aperture there are electrodes between which flows the direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As the current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell size is plotted by determining the pulse size. Also analyzing a histogram makes it possible to obtain various analysis data.

To analyze the haemoglobin by automated methods, the cyanmethaemoglobin or oxyhaemoglobin methods have so far been the mainstream.

Serum Iron level assay:

Method: Ferrozine method without deproteinization.

Serum Iron binding capacity:

Method: Spectrophotometric assay.

Ferritin estimation:

Method: Fully automated biodirectionally interfected chemiluminescent immuno assay.

PRINCIPLE OF IRON & TIBC ESTIMATION:

Iron bound to transferrin, is released in an acidic medium and the ferric ions are reduced to ferrous ions, the Fe(II) ions react with ferrozine to form a violet coloured complex. Intensity of the complex formed is directly proportional to the amount of Iron present in the sample.

For TIBC, the serum is treated with excess of Fe(II) to saturate the Iron binding sites on transferrin. The excess Fe(II) is adsorbed and precipitated and the Iron content in the supernatant is measured by spectrophotometry to give the TIBC.

$$\text{Fe(III)} \xrightarrow{\text{Acidic medium}} \text{Fe(II)}$$

$$\text{Fe(II)} + \text{Ferrozine} \longrightarrow \text{violet coloured complex}$$

RED CELL INDICES:

1. **Mean corpuscular volume (MCV) in femtolitres**

$$\text{MCV} = \frac{\text{Packed cell volume in \%}}{\text{Red cell count in millions/cmm}} \times 10$$

2. **Mean corpuscular haemoglobin (MCH) in picograms**

$$\text{MCH} = \frac{\text{Haemoglobin (gm/dl)}}{\text{Red cell count in millions/cmm}} \times 10$$

3. Mean corpuscular haemoglobin concentration (MCHC) in gm/dl

$$MCHC = \frac{\text{Haemoglobin (gm/dl)}}{\text{Packed Cell Volume (\%)}} \times 100$$

4. Reticulocyte count:

$$\text{Reticulocyte count} = \frac{\text{Reticulocyte counted}}{\text{Number of red cells}} \times 100$$

Following indices were also obtained for these patients

Red Blood Cell (RBC) count

RBC Distribution Width (RDW)

$$\textbf{Mentzer index} = \frac{\text{Mean cell volume}}{\text{Red blood cell count}}$$

Shine and Lal Index = Mean cell volume ^{2} X Mean corpuscular Haemoglobin X 0.01

England & Fraser Index =Mean cell volume-Red blood cell-5Hb-3Hb

$$\textbf{Srivasthava index} = \frac{\text{Mean corpuscular Hb}}{\text{Red blood cell}}$$

$$\textbf{Green \& king index} = \frac{\text{Mean cell volume}^2 \text{ X RDW}}{100 \text{ Hb}}$$

$$\textbf{RBC Distribution width index} = \frac{\text{Mean cell volume X RDW}}{\text{RBC}}$$

$$\textbf{Ricerca index} = \frac{\text{Red cell distribution width}}{\text{Red blood cell count}}$$

Threshold values of Indices used to discriminate between Iron deficiency anaemia and β–Thalassemia Trait

Indices	IDA	β - TT
Ricerca Index	>4.4	<4.4
RBC count	<5	>5
RBC distribution width	>14	<14
Mentzer index	>13	<13
Shine and Lal index	>1530	>1530
England and Fraser Index	Positive	Negative
Srivastava Index	>3.8	<3.8
Green and King Index	>65	<65
RBC distribution width index	>220	<220

RED CELL DISTRIBUTION WIDTH:

This is an quantitative measurement of variation in cell volume, that is percentage of cells falling above and below Mean cell volume. Actually it is microscopic equivalent to degree of anisocytosis. RDW is derived from the pulse height analysis and can be expressed as standard deviation (in fl) or as coefficient of variation (cv%). **Reference value:** SD: 42. 5 (+/_) 3.5 fl.

OBSERVATION AND RESULTS

Newly diagnosed cases of microcytic hypochromic anaemia admitted to Coimbatore Medical College hospital with no prior blood transfusion or iron treatment were randomly enrolled. A total of 44 cases were enrolled as per inclusion and exclusion criteria.

AGE:

Patient with Microcytic Hypochromic Anaemia with age between 1-12 years were included in this study. Out of 44 cases, 27.3% were below 4 years, 36.4% were between 5-8 years & 36.4% were between 8-12 years.

Table-1: **Age incidence**

Age (in years)	Study population	Percent
1-4	12	27.3
5-8	16	36.4
9-12	16	36.4
Total	44	100.0

[n=Total number of cases (44)]

SEX

Among the randomly enrolled 44 patients, 25 were girls while the remaining 19 were boys. Girls constituted 56.82%, boys constituted 43.18%.

SEVERITY OF ANAEMIA

Mild anaemia (Hb 10-12 g/dl) was present in 26 cases, moderate anaemia (Hb 8-9.9 g/dl) in 13 cases and severe anaemia (Hb <8 g/dl) in 5 cases.

Table-2: Severity of anaemia:

Hb (g/dl)	Study population	Percent
< 8	26	59.1
8 – 9.9	13	29.5
10 – 11	5	11.4
Total	44	100.0

MEAN CORPUSCULAR VOLUME (MCV)

97.7% of study population had MCV below 80 fl.

Table-3: MCV distribution

MCV(fl)	Study population	Percent
<80	43	97.7
80 – 100	1	2.3
Total	44	100.0

MEAN CORPUSCULAR HAEMOGLOBIN

93.2% had MCH below 25pg.

Table-4: MCH distribution

MCH (pg)	Study population	Percent
25 & below	41	93.2
26 – 34	3	6.8
Total	44	100.0

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION

95.5% had MCHC below 30 g/dl.

Table-5: MCHC distribution

MCHC (g/dl)	Study population	Percent
30 & below	42	95.5
31 – 37	2	4.5
Total	44	100.0

SERUM FERRITIN:

Out of 44 cases, 52.3% had S. Ferritin below 12ng/dl, 20.5 % had S. Ferritin between 12-50ng/dl & 27.3% had S. Ferritin above 50ng/dl.

Table-6: Serum Ferritin

S. Ferritin (ng/dl)	Study population	Percent
<12	23	52.3
12-50	9	20.5
>50	12	27.3
Total	44	100.0

TOTAL IRON BINDING CAPACITY:

Out of 44 cases, 6.8% had TIBC < 250 µg/dl, 45.5% had TIBC between 250-400 µg/dl & 47.7% had TIBC >400 µg/dl.

Table-7: Total Iron binding capacity (TIBC)

TIBC (µg/dl)	Study population	Percent
<250	3	6.8
250-400	20	45.5
>400	21	47.7
Total	44	100.0

SERUM IRON IN FEMALE CHILDREN:

Out of 25 girls in the study population, 80% had S. Iron < 60 µg/dl, 4% had S. Iron 60- 160 µg/dl & 16% had S. Iron >160 µg/dl.

Table-8: Serum Iron in girls

S. Iron (µg/dl)	No. of girls in the study	Percent
<50	20	80.0
50-150	1	4.0
>150	4	16.0
Total	25	100.0

SERUM IRON IN MALE CHILDREN:

89. 5% of boys in the study population had a S. Iron below 35µg/dl.

Table-9: Serum Iron in boys

S. Iron (µg/dl)	No. of boys in the study	Percent
<50	18	94.7
50-150	-	-
>150	1	5.3
Total	19	100.0

AGE WISE STATISTICAL ANALYSIS

Mean haemoglobin level does not vary significantly with age. When ANOVA test was conducted (F=2.765; P>0.05) the mean haemoglobin was highest in the age group of 9- 12 years (7.76 g/dl) and lowest in the age group of 1-4 years (5.93 g/dl).

Table-10: Haemoglobin in various age groups

Age (in years)	Haemoglobin (g/dl) Mean	S.D	Study population
1 – 4	5.93	2.08	12
5 – 8	6.33	2.13	16
9 – 12	7.76	2.39	16
Total	6.74	2.31	44

RBC COUNT IN VARIOUS AGE GROUPS

Mean RBC counts varied significantly with age. When ANOVA test was conducted (F= 4.799; P<0.05*) the mean RBC count was highest in the age group of 9- 12 years (3.81million/ mm^3) and lowest in the age group of 5-8 years (2.73 million/mm^3). (*- significant at 5% level)

Table-11: RBC count in various age groups

Age (in years)	RBC count (million/mm^3) Mean	S.D	Study population
1-4	2.82	0.81	12
5-8	2.73	0.96	16
9-12	3.81	1.31	16
Total	3.15	1.16	44

[SD= Standard deviation**]**

HAEMATOCRIT IN VARIOUS AGE GROUPS

Mean haematocrit does not vary significantly based on age. When ANOVA test was conducted (F= 3.098 ; P>0.05) the mean haematocrit was highest in the age group of 9- 12 years (22.33%) and lowest in the age group of 5-8 years (17.10%).

Table-12: Haematocrit (HCT**)** in various age groups

Age (in years)	HCT (%) Mean	S.D	Study population
1 – 4	17.59	6.96	12
5 – 8	17.1	6.3	16
9 – 12	22.33	6.2	16
Total	19.13	6.76	44

MEAN CORPUSCULAR VOLUME IN VARIOUS AGE GROUPS

Mean MCV varied significantly with age. When ANOVA test was conducted (F= 0.069; P < 0.05*) the MCV was highest in the age group 5-8 years (62.25 fl) and lowest in the age group of 1-4 years(60.75 fl).

Table-13: Mean corpuscular volume in various age groups

Age (in years)	MCV (fl) Mean	S.D	Study population
1 – 4	60.75	9.93	12
5 – 8	62.25	9.5	16
9 – 12	61.19	13.28	16
Total	61.46	10.9	44

MCH IN VARIOUS AGE GROUPS

Mean MCH does not vary significantly with age. When ANOVA test was conducted (F= 0.250; P>0.05) the mean MCH was highest in the age group of 5-8 years (19.27pg) and lowest in the age group of 1-4 years (17.99 pg).

Table-14: Mean corpuscular haemoglobin in various age groups

Age (in years)	MCH (pg) Mean	S.D	Study population
1-4	17.99	5.18	12
5-8	19.27	4.68	16
9-12	18.61	4.57	16
Total	18.68	4.69	44

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION IN VARIOUS AGE GROUPS

Mean MCHC does not vary significantly based on age. When ANOVA test was conducted (F= 0.757; P >0.05) the mean MCHC was highest in the age group 9-12years (25.05g/dl) and lowest in the age group of 1-4 years (23.20g/dl).

Table-15: Mean corpuscular haemoglobin concentration in Various age groups

Age (in years)	MCHC (g/dl) Mean	S.D	Study population
1-4	23.20	2.00	12
5-8	23.78	4.95	16
9-12	25.05	4.40	16
Total	24.08	4.12	44

RED CELL DISTRIBUTION WIDTH IN VARIOUS AGE GROUPS

Mean RDW varied significantly based on age. When ANOVA test was conducted (F= 4.961; P<0.05*) the mean RDW was highest in the age group 1-4 years (53.01fl) and lowest in the age group of 9-12 years (44.15fl).

Table-16: Red cell distribution width in various age groups

Age (in years)	RDW (fl) Mean	S.D	Study population
1-4	53.01	9.94	12
5-8	50.68	8.14	16
9-12	44.15	5.51	16
Total	48.94	8.56	44

SERUM IRON IN VARIOUS AGE GROUPS

Mean S. Iron does not vary significantly with age. When ANOVA test was conducted (F= 0.217; P>0.05) the mean Iron was highest in the age group of 1-4 years (66.40 gμ/dl) and lowest in the age group of 5-8 years (45.99μg/dl)

Table-17: Serum Iron in various age groups

Age (in years)	Serum Iron(μg /dL) Mean	S.D	Study population
1-4	66.40	96.57	12
5-8	45.99	80.07	16
9-12	52.61	70.98	16
Total	53.96	80.30	44

TIBC IN VARIOUS AGE GROUPS

Mean TIBC does not vary significantly based on age. When ANOVA test was conducted (F= 0.569; P>0.05) the mean TIBC was highest in the age group 5-8 years (402.53µg/dl) and lowest in the age group of 9-12 years (372.40 µg/dl). **Table-18: TIBC in various age groups**

Age (in years)	TIBC (µg /dl) Mean	S.D	Study population
1-4	380.18	109.17	12
5-8	402.53	68.33	16
9-12	372.20	73.17	16
Total	385.40	82.01	44

FERRITIN IN VARIOUS AGE GROUPS

Mean ferritin does not vary significantly based on age. When ANOVA test was conducted (F= 0.928; P>0.05) the mean ferritin was highest in the age group of 1-4 years (195.15ng/dl) and lowest in the age group of 5-8 years (43.30ng/dl). **Table-19:** Ferritin in various age groups

Age (in years)	Ferritin (ng /dl) Mean	S.D	Study population
1-4	195.15	533.78	12
5-8	43.30	83.58	16
9-12	104.26	134.00	16
Total	106.88	292.01	44

GENDERWISE STATISTICAL ANALYSIS

Mean Haemoglobin level does not vary significantly between boys and girls. When t-test was applied (t= 0.087; P>0.05) the average haemoglobin in boys was 6.77g/dl & for girls 6.71g/dl.

Table-20: Distribution of Haemoglobin in both sexes

Gender	Haemoglobin (g/dl) Mean	S.D	Study population
Girls	6.71	2.32	25
Boys	6.77	2.35	19
Total	6.74	2.31	44

DISTRIBUTION OF RBC COUNT IN BOTH SEXES

Mean RBC counts do not vary significantly between boys and girls. When t- test was applied (t=1.707; P>0.05) the average RBC count in girls was 3.40 million/mm^3 where as for boys it was 2.81 million/mm^3

Table-21: Distribution of RBC count in both sexes

Gender	RBC count (million/mm^3) Mean	S.D	Study population
Girls	3.40	1.38	25
Boys	2.81	0.68	19
Total	3.15	1.16	44

DISTRIBUTION OF HAEMATOCRIT IN BOTH SEXES

Mean Haematocrit does not vary significantly between boys and girls. When t- test was applied (t= 0.559; P>0.05) the average HCT in girls was 19.63% where as for boys it was 18.47%.

Table-22: Distribution of Haematocrit in both sexes

Gender	HCT (%) Mean	S.D	Study population
Girls	19.63	7.58	25
Boys	18.47	5.64	19
Total	19.13	6.76	44

DISTRIBUTION OF MCV IN BOTH SEXES

MCV does not vary significantly between boys and girls. When t- test was applied (t=1.985; P>0.05) the average MCV in boys was 65.07fl where as for girls it was 58.71fl.

Table-23: Distribution of MCV in both sexes

Gender	MCV (fl) Mean	S.D	Study population
Girls	58.71	11.75	25
Boys	65.07	8.68	19
Total	61.46	10.90	44

DISTRIBUTION OF MCH IN BOTH SEXES

Mean MCH does not vary significantly between boys and girls. When t- test was applied (t= 0.256; P>0.05) the average MCH in girls was 18.52pg where as for boys it was 18.89 pg.

Table-24: Distribution of MCH in both sexes

Gender	MCH (pg) Mean	S.D	Study population
Girls	18.52	4.77	25
Boys	18.89	4.71	19
Total	18.68	4.69	44

DISTRIBUTION OF MCHC IN BOTH SEXES

Mean MCHC does not vary significantly between boys and girls. When t- test was applied (t=0.556; P>0.05) the average MCHC in girls was 24.39g/dL where as for boys it was 23.68 g/dl.

Table-25: Distribution of MCHC in both sexes

Gender	MCHC (g/dl) Mean	S.D	Study population
Girls	24.39	4.08	25
Boys	23.68	4.24	19
Total	24.08	4.12	44

DISTRIBUTION OF RDW IN BOTH SEXES

Mean RDW does not vary significantly between girls and boys. When t- test was applied ($t=1.067$; $P>0.05$) the average RDW in girls was 47.74fl where as for boys it was 50.52 fl.

Table-26: Distribution of RDW in both sexes

Gender	RDW (fl) Mean	S.D	Study population
Girls	47.74	9.03	25
Boys	50.52	7.84	19
Total	48.94	8.56	44

DISTRIBUTION OF SERUM IRON IN BOTH SEXES

Mean serum Iron does not vary significantly between boys and girls. When t- test was applied ($t=1.575$; $P>0.05$) the average serum Iron in girls was 70.31µg/dl where as for boys it was 32.46 µg/dl.

Table-27: Distribution of Serum Iron in both sexes

Gender	Serum Iron (µg/dl) Mean	S.D	Study population
Girls	70.31	98.31	25
Boys	32.46	40.69	19
Total	53.96	80.30	44

DISTRIBUTION OF TIBC IN BOTH SEXES

Mean TIBC does not vary significantly between boys and girls. When t- test was applied (F=1.079; P>0.05) the average serum Iron in girls was 373.79 µg/dl where as for boys it was 400.68 µg/dl.

Table-28: Distribution of TIBC in both sexes

Gender	TIBC (µg/dl) Mean	S.D	Study population
Girls	373.79	90.81	25
Boys	400.68	68.12	19
Total	385.40	82.01	44

DISTRIBUTION OF FERRITIN IN BOTH SEXES

Mean ferritin does not vary significantly between boys and girls. When t- test was applied (t=0.796; P>0.05) the average ferritin in girls was 141.87 ng/dl where as for boys it was 70.81 ng/dl.

Table-29: Distribution of ferritin in both sexes

Gender	Ferritin (ng/dl) Mean	S.D	Study population
Girls	141.87	370.69	25
Boys	70.81	131.43	19
Total	111.19	291.88	44

STATISTICAL ANALYSIS BETWEEN β –TT AND MILD TO MODERATE (8-11.5 G/DL) IDA

Mean haemoglobin varied significantly between IDA, β-TT. When t-test was applied (t=3.239; P<0.01) the mean Haemoglobin in IDA patients was 8.99g/dl where as for β-TT 10.57g/dl.

Table-30: Distribution of Haemoglobin in IDA & β-TT

Anaemia	Haemoglobin(g/dL) Mean	S.D	Mild to moderate anaemia
IDA	8.99	.81	11
B- TT	10.57	.23	3
Total	9.33	.98	14

DISTRIBUTION OF MCV IN IDA & β-TT

Mean MCV varied significantly between IDA, β-TT. When t-test was applied (t=5.402; P<0.01**) the mean MCV in IDA patients was 68.45 (fl) where as for β-TT 38.91(fl). (**- significant at 1% level)

Table-31: Distribution of MCV in IDA & β-TT

Anaemia	MCV (fl) Mean	S.D	Mild to moderate anaemia
IDA	68.45	9.20	11
β- TT	38.91	.16	3
Total	62.12	14.95	14

DISTRIBUTION OF MCH IN IDA & β-TT

Mean MCH do not vary significantly between IDA, β-TT. When t-test was applied (t=1.938; P>0.05) the mean MCH in IDA patients was 20.79 pg where as for β-TT 17.04 pg.

Table-32: Distribution of MCH in IDA & β-TT

Anaemia	MCH (pg) Mean	S.D	Mild to moderate anaemia
IDA	20.79	3.24	11
B- TT	17.04	.80	3
Total	19.99	3.27	14

DISTRIBUTION OF MCHC IN IDA & β-TT

Mean MCHC do not vary significantly between IDA, β-TT. When t-test was applied (t=1.382; P>0.05) the mean MCHC in IDA patients was 23.83g/dl where as for β-TT 27.52 g/dl.

Table-33: Distribution of MCHC in IDA & β-TT

Anaemia	MCHC (g/dl) Mean	S.D	Mild to moderate anaemia
IDA	23.83	4.49	11
B- TT	27.52	.54	3
Total	24.62	4.25	14

DISTRIBUTION OF RDW IN IDA & β-TT

Mean RDW varied significantly between IDA, β-TT. When t-test was applied (t=5.407; P<0.01) the mean RDW in IDA patients was 47.77 fl where as for β-TT 35.02 fl.

Table-34: Distribution of RDW in IDA & β-TT

Anaemia	RDW (fl) Mean	S.D	Mild to moderate anaemia
IDA	47.77	3.66	11
B-TT	35.02	3.42	3
Total	45.04	6.45	14

DISTRIBUTION OF SERUM IRON IN IDA & β-TT

Mean serum Iron does not vary significantly between IDA, β-TT. When t-test was applied (t=1.248; P> 0.05) the mean serum Iron in IDA patients were 50.11 µg/dl whereas for β-TT 131.03 µg/dl.

Table-35: Distribution of S.Iron in IDA & β-TT

Anaemia	Serum Iron (µg/dl) Mean	S.D	Mild to moderate anaemia
IDA	50.11	92.19	11
β-TT	131.03	130.36	3
Total	67.45	101.68	14

DISTRIBUTION OF TIBC IN IDA & β-TT

Mean TIBC varied significantly between IDA, β-TT. When t-test was applied (t=3.115; P<0.01) the mean TIBC in IDA patients were 424.87µg/dl whereas for β-TT 322.42 µg/dl.

Table-36: Distribution of TIBC in IDA & β-TT

Anaemia	TIBC(µg/dl) Mean	S.D	Mild to moderate anaemia
IDA	424.87	52.65	11
β-TT	322.42	37.97	3
Total	402.92	65.25	14

DISTRIBUTION OF SERUM FERRITIN IN IDA & β-TT

Mean serum ferritin varied significantly between IDA, β-TT. When t-test was applied (t= 22.800; P<0.01) the mean serum ferritin in IDA patients were 12.51 ng/dl whereas for β-TT 206.63 ng/dl

Table-37: Distribution of Serum Ferritin in IDA & β-TT

Anaemia	Ferritin (ng/dl) Mean	S.D	Mild to moderate anaemia
IDA	12.51	8.01	11
B-TT	206.63	26.54	3
Total	54.11	83.61	14

DISCUSSION

The current study mainly focused on the utility of serum iron profile, hemoglobin electrophoresis and peripheral smear in microcytic hypochromic anaemia. This study also attempted to elucidate the diagnostic accuracy of seven indices to discriminate mild to moderate Iron deficiency from

β - Thalassemia.

AGE, SEX INCIDENCE & SEVERITY OF ANAEMIA:

The present study included patients between the age group of 1- 12 years. Two thirds of anaemic children were between the age of 5 & 12 years. This could be attributed to the increasing nutritional demands of growth spurt and puberty compounded by less attention to nutrition, amidst demanding academic pressures.

In the present study, girls with microcytic hypochromic anaemia out number the boys. This female preponderance could be due to less care of the girl child in Indian settings. Distribution of Hb, RBC, HCT, MCV, MCH, MCHC, RDW do not vary significantly in both sexes.

Mild anaemia (Hb10- 12 g /dl) was present in 26 cases (11.4%), moderate anaemia (Hb 8 -9.9g/dl) in 13 cases (29.5%) and severe anaemia (Hb <8 g/dl) in 5 cases (59.1%).

PREVALENCE OF IDA:

A study conducted by **Mohemmed et al.** in the 2006 **& Looker et al.** [37] in 1997, found that IDA is most prevalent in children.

This finding correlates with the present study where 72.73% of microcytic hypocromic anaemia was found to be due to Iron deficiency.
[β-Thalassemia trait; 6.82%, β-Thalassemia major; 6.882% & anaemia of chronic disease; 13.64%].

This is an expected observation as early childhood represents a period of rapid growth and depletion of blood iron. (**Looker et al.**[37]). On the other hand, adolescent girls are also more susceptible to iron deficiency because of poor dietary intake in conjunction with high iron requirements related to rapid growth and menstrual blood loss. This study reflects similar findings probably due to the same factors.

VALUE OF RED CELL INDICES IN DIFFERENTIATING BETWEEN BETA-TT & IDA:

Mean haemoglobin varied significantly between IDA, β-TT. The mean Hemoglobin in IDA patient was 8.99g/dL whereas for β-TT 10.57g/dL. Mean MCV varied significantly between IDA, β-TT, mean MCV in IDA patient was 68.45 (fl) whereas for β-TT 38.91 (fl).

MCV is known to be significantly low in β-Thalassemia as compared to Iron deficiency anaemia. Mean MCH does not vary significantly between IDA, β-TT, mean MCH in IDA patient was 20.79 pg whereas for β-TT 17.04 pg. Mean MCHC does not vary significantly between IDA, β-TT, mean MCHC in IDA patient was 23.83g/dl whereas for β-TT 27.52 g/dl.

RED CELL INDICES AND ITS USES:

A study conducted by **M.A.Ehani et al.**[38] included 284 patients aged (range 10 – 38 years), this study utilized 4 indices including England and Fraser Index , Mentzer Index, Srivastava index & RBC count to discriminate 130 cases of IDA & 154 cases of b TT. Youden's index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity [33,34], and was first used by **Demir et al.**[38] Youdens index was calculated, showed MI (90.1) to be superior to Srivastava index (74.2) and England & fraser index(68.7) in that order.

Table-38: Following table shows sensitivity, specificity & Youden's index calculated by **Ehani et al.**[38]

Indices	Sensitivity (%)	Specificity (%)	Youden's index
England &Fraser Index IDA B –TT	99.2 69.5	69.5 99.2	68.7
Mentzer Index IDA B-TT	94.6 95.5	95.5 94.6	90.1
Srivastava index IDA B-TT	88.5 85.7	85.7 88.5	74.2
RBC count IDA B= TT	86.2 98.1	98.1 86.2	84.3

According to another study conducted by **Damier et al.**[38] sample size included was 63, best two indices were RBC count & RDWI. Youden's index calculated was 82 & 80 respectively.

A study conducted by **Ntaios et al.**[40] (2007), sample size included were 493, it was concluded that best two indices was Green and King, England and Fraser index, Youden's index calculated was 70.9 , 63.2 respectively.

A study conducted in the year 2007 by **Beyan et al.**[41] included a sample size of 111; it was concluded that best two indices were RBC count & Green and king indices, Youden's index calculated was 73.7& 65.5 respectively.

A study conducted in the year 2008 by **Sirdah et al**[42] included a sample size of 2196; it was concluded that best two indices were Green and king indices, Red cell distribution width index, Youden's index calculated was 68.6 & 68.4 respectively.

Another study conducted by **Urrechaga et al** (2008)[43], sample size included were 318, they come to a conclusion that best indices was Green and King, Youden's index calculated was 80.9.

Table-39: Youden's index in various studies:

References	Sample size	Best indices	Youden' index
Damier et al. (2002)	63	RBC count RDWI	82 80
Ntaios et al.(2007)	493	Green & king England & Fraser	70.9 63.2
Beyan et al. (2007)	111	RBC count Green & King	73.7 65.5
Sirdah et al.(2008)	2196	Green &King RDWI	68.6 68.4
Urrechaga et al. (2008)	318	Green & King	80.9
Ehani et al.	284	Mentzer	90.1

The S&L index was first defined by Shine and Lal in 1977 and was reported to have a sensitivity of 100%, a specificity of 11.8%and an efficiency value of 59.5% for differentiating between β-TT and IDA patients.

Yeo et al.[44]. found the S&L index and mean cell volume (MCV) to be applicable when applied to pregnant women in Singapore.

Lafferty et al.[45]found the S&L index, MI and MCV to be valuable in distinguishing IDA and β-TT minor cases; the RDW and the E&F indices were not useful.

In studies including schoolchildren in Jordan, MI, MCV ≤72 fl, and E&F and S&L indices correctly identified 91.6%, 82.4%, 81.3% and 62.6%, respectively, of microcytosis cases as having or not having the β-TT trait12.

AlFadhli et al.[46] found the E&F index to be the most discriminatory and the S&L index the least when comparing patients with IDA to those with β-TT or TT minor.

A study conducted by **Okan et al.**[47] found the S&L and G&K indices to be best at differentiating IDA from β-TT patients, and the RDW index to be the worst. In particular, the S&L index had the highest Youden index value in discriminating β-TT cases from those with moderate-to-severe IDA and also from those with mild IDA.

Table-40: Following table shows sensitivity, specificity, positive predictive value, negative predictive value & Youden's index calculated in **Okans et al** study

Indices	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
Red blood cell count IDA B TT	75 86	86 75	84.2 77.4	77.4 84.2	61
Red cell distribution width IDA b- TT	98 6	6 98	51 75	75 51	4
Mentzer index IDA B-TT	78 82	82 78	81.2 78.8	78.8 81.2	60
Shine and Lal index IDA b-TT	100 91	91 100	91.7 100	100 91.7	91
England & Fraser index IDA B –TT	97 78	78 97	81.5 96.2	96.2 81.5	75
Srivastava index IDA b- TT	79 74	74 79	75.2 77.8	77.8 75.2	53
Green & king index IDA b- TT	96 83	83 96	84.9 95.4	95.4 84.9	79
RDW index IDA b-TT	78 83	83 78	82.1 87.3	87.3 82.1	61
Ricerca index IDA B-TT	14 98	98 14	87.5 53.2	53.2 87.5	12

Table-41: Following table shows sensitivity, specificity, Positive predictive value, negative predictive value found in the present study:

Indices	Anaemia	Sensitivity	Specificity	PPV	NPV	Y.I
Mentzer index	IDA	100.0	100.0	100.0	100.0	100.0
	B-TT	100.0	100.0	100.0	100.0	
Shine and Lal Index	IDA	9.1	100.0	100.0	23.1	9.1
	B-TT	100.0	9.1	23.1	100.0	
England Fraser Index	IDA	100.0	100.0	100.0	100.0	100.0
	B-TT	100.0	100.0	100.0	100.0	
Srivasthava index	IDA	100.0	100.0	100.0	100.0	100.0
	B-TT	100.0	100.0	100.0	100.0	
Green & king index	IDA	100.0	100.0	100.0	100.0	100.0
	B-TT	100.0	100.0	100.0	100.0	
RBC Distribution width index	IDA	100.0	33.3	84.6	100.0	33.3
	B-TT	33.3	100.0	100.0	84.6	
Ricerca index	IDA	100.0	0.0	78.6	0.0	0.0
	B-TT	0.0	100.0	0.0	78.6	

Compared to the previous study, present study showed 100% sensitivity, specificity in 4 indices. This could be due to the small sample size in the present study.

The present study found that, to differentiate mild to moderate IDA (Hb 8.5 – 11 g/dl) from β-TT Mentzer index, England and Fraser index, Srivastava index & Green and king index had highest specificity as well as Youden's index. However RBC distribution width index was found to have reasonable specificity and sensitivity when compared to Shine and lal index & Ricerca index.

Table- 42: Hematological parameter

Parameter	Ehana et al.		Present study	
	IDA	B-TT	IDA	b-TT
Hb (g/dl)	9.3(+/-)1.89	11.24(+/-) 1.37	8.99(+/-)0.81	10.57(+/-) 0.23
MCV (fl)	70.04(+/-) 7.94	62.02 (+/-) 4.57	68.45(+/-) 9.20	38.91(+/-) 0.16
MCH (pg/cell)	21.30 (+/-) 3.52	19.68 (+/-) 1.53	20.79(+/-) 3.24	17.04(+/-) 0.8
MCHC (g/dl)	29.88(+/-) 2.86	30.93(+/-) 1.71	23.83(+/-) 4.49	27.52(+/-) o.54

A study conducted by **Ehana et al.**[38] found that MCHC was low in Iron deficiency anaemia, whereas MCV, MCH were low in Thalassemia & MCHC was normal. The present study showed similar results.

SUMMARY AND CONCLUSION

- In the present study of 44 children with microcytic hypochromic anaemia 12 were in 1-4 year age group (27.3%), 16 were in 5-8 year age group (36.4%) & 16 were in 9-12year age group (36.4 %).

- Out of 44 patients studied, 56.8% were girls, 43.2% were boys.

- Mild anaemia was observed in 11.4% of children; moderate anaemia was observed in 29.5 % of children, severe anaemia was observed in 59.1% of cases.

- Out of 44 cases, IDA constituted 72.73%, β-Thalassemia trait constituted 6.82%, β-Thalassemia major constituted 6.882% & Anaemia of chronic disease constituted 13.64%.

- Following median values were obtained:

For IDA- RBC count- 2.58million/mm^3, Hemoglobin- 5.85g/dl, MCH- 19.2 pg, MCHC- 22.45g/dl, RDW- 50.4 fl, S.Iron- 22µg/dl, Serum Ferritin- 4.67ng/dl & TIBC- 411.35µg/dl.

- β Thalassemia trait: -

RBC count 5.36 million/mm^3, MCV-62.1fl, MCH- 20.6 pg, MCHC-22.1g/dl, S.Iron-199.8µg/dl, Serum Ferritin-307.3ng/dl & TIBC-267.1µg/dl.

- Mean haemoglobin, MCV, MCH, RDW were lower in boys. Mean RBC, MCHC, HCT were higher in girls. Mean Serum Iron & Ferritin were low and TIBC was high in girls.

- Out of 44 cases, 14 with mild to moderate anaemia caused clinical confusion between Iron deficiency anaemia and β-Thalassemia trait. After doing Iron profiles and electrophoresis 11 were concluded as Iron deficiency anaemia, and 3 were concluded as β-Thalassemia trait.

- In remaining 30 cases, 3 turned out to β-Thalassemia major and 6 were found to be anaemia of chronic disease.

- In 6 children with anaemia of chronic disease, 3 were suffering from pneumonia, other two were suffering from urinary tract infection and remaining one child was suffering from tuberculosis.

- MCV was found to be lower in β-Thalassemia Trait than in IDA, which could have a useful application in differentiating these two conditions.

- 7 indices were used to differentiate mild to moderate Iron deficiency anaemia and β-Thalassemia trait. For differentiating these two entities, Youdens index showed that Mentzer index, England and Fraser index, Srivastava index & Green and King index were equally superior to RBC Distribution width index, Shine and lal index & Ricerca index in that order.

- In microcytic hypochromic anaemia peripheral smear, red cell indices, Serum Iron profile including Serum Iron, TIBC, Serum Ferritin and Haemoglobin electrophoresis were found to be useful parameters in the precise assessment of anaemia and its type.

- **Hb Electrophoresis, Serum Iron profile & Red cell indices are complementary to each other in the precise diagnosis of microcytic hypochromic anaemia of varied etiology, which would enable comprehensive wholesome treatment.**

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ANNEXURE I

PROFORMA

Name

Age

Sex

Residential address

Informant

Reliability of Informant

Education of Informant

Occupation

Consanguinity

Family history

Birth and developmental history

Order of birth

Spacing of birth

Clinical history

Fatigability

Irritability

Poor weight gain

Breathlessness on exertion

H/O Passing worms in stool / Vomitus

H/O Bleeding tendencies

H/O Bone tenderness

H/O Recurrent urinary tract infection

H/O Chronic leg ulcers

H/O Jaundice

H/O Pica

Chronic drug intake

Having gone to endemic area for malaria during previous eight weeks

Menstrual history

H/O menorrhagia

Physical examination

Pallor/Jaundice

Koilonychia

Hepatomegaly/ Splenomegaly

Hepatosplenomegaly

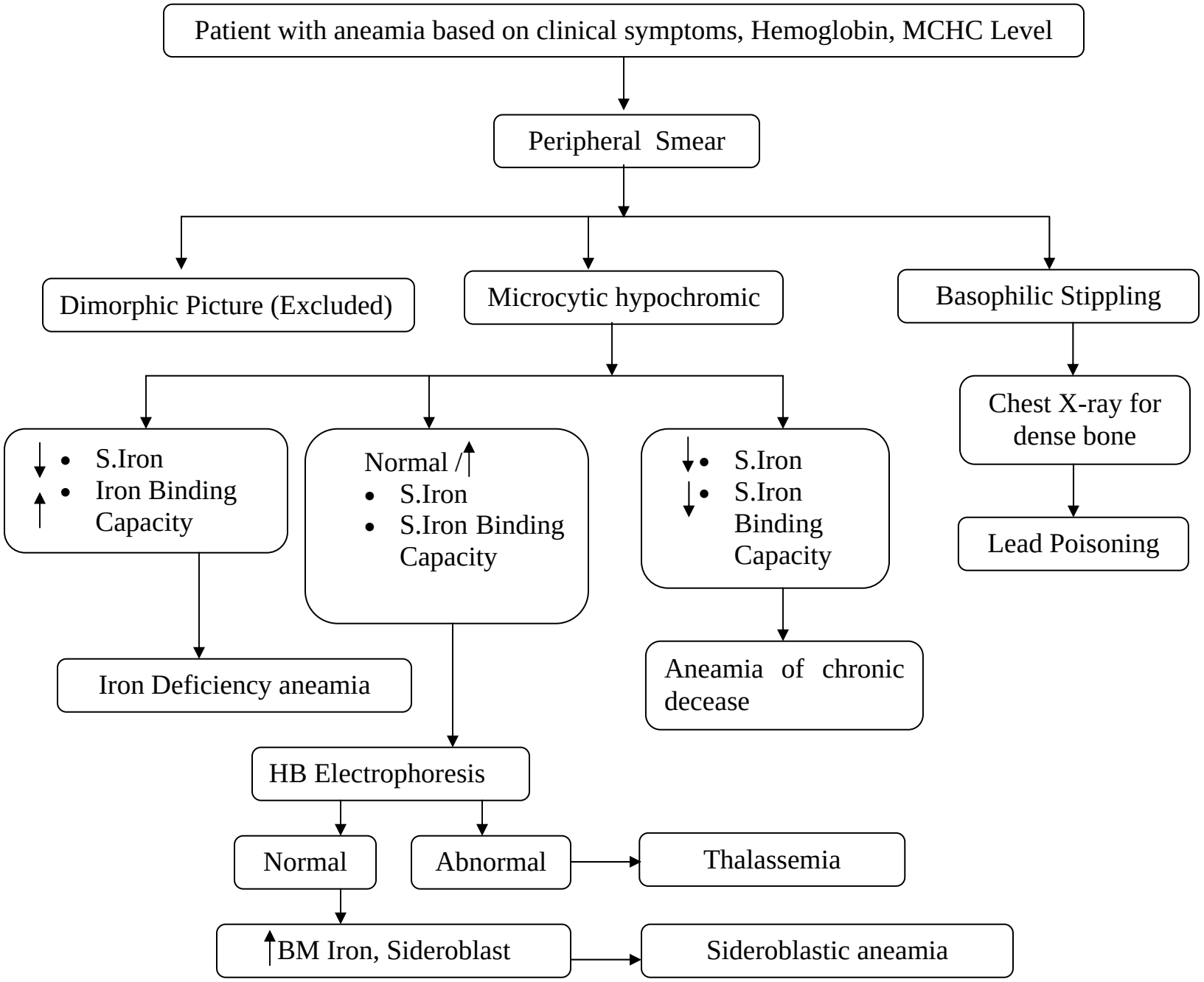
Skin purpura/ petechia,

Tachypnea/ Dyspnea

Pedal edema

Raised JVP

ANNEXURE-2: METHODOLOGY



METHOD

1. Patients with anaemia based on the clinical signs and symptoms were selected.
2. They were tested for haemoglobin level, Mean corpuscular haemoglobin value, Mean corpuscular haemoglobin Concentration, Mean corpuscular volume.
3. Peripheral smears were examined.
4. With the help of these profiles patients were classified as microcytic hypochromic anaemia and included in the study.
5. Patients with macrocytic and dimorphic blood picture were excluded.
6. Patients with hypochromic microcytic anaemia were analyzed for Serum Iron, Serum Iron binding capacity, Serum ferritin, transferrin saturation.
7. If Serum Iron level, ferritin were decreased and Iron binding capacity increased, that condition was categorized as Iron deficiency anaemia.
8. If Serum Iron, ferritin were increased, Iron binding capacity was decreased - it indicates increased iron stores. Haemoglobin electrophoresis was done to rule out/ confirm Thalassemia trait.
9. If electrophoresis showed increased Hb A2 it indicated Thalassemia minor. If it showed increased Hb F it indicated Thalassemia major

10. If Serum Iron, Serum Iron binding capacity were decreased, Serum ferritin was increased it was categorized as anaemia of chronic disease.
11. If smear showed basophilic stippling, possibility of lead poisoning was considered and X ray for bone density was done.

ANNEXURE-3

MASTER CHART: 1

Sl	IP No	Age	Sex	Hb (g/dl)	MCV (fl)	MCH (Pg)	MCHC (g/dl)	RBC (µ/dl)	HCT (%)	RDW	S.Iron(µg/dl)	TIBC (µg/dl)	Ferritin (ng/dl)	Transferrin saturation (%)	Hb Electrophoresis			Result
															Hb A	Hb A2	Hb F	
1	40764-09	5	F	4.1	77.4	25.6	22	3.21	24.85	53.5	22	480	0.5	4.58				IDA
2	44992-09	3	M	3.5	76	24.8	22.7	2.7	20.52	50.4	22	444.9	2.74	4.97				IDA
3	48837-09	12	M	8.7	79.4	24.5	27.4	2.59	20.56	51.7	22	395.8	20.26	6.97				IDA
4	56630-09	1	F	8.3	69.3	24	22.7	3.77	26.13	47.3	28.2	357.7	27.2	7.88				IDA
5	56888-09	10	F	4.5	58.1	12.5	21.5	2.51	14.58	48.1	23	412.4	6.96	5.58				IDA
6	56960-09	6	F	5.8	56.4	20.6	30.5	5.62	31.70	41.7	345	200.8	285.5	86.08	8.3	2.5	89.2	β-TT
7	56963-09	7	F	8.1	70.1	19.1	27.2	3.21	22.50	41.7	44.4	350.7	200.8	12.66				CD
8	64184-09	8	F	4.9	47.4	10.7	22.6	1.58	7.49	63	38.2	405.9	51.56	9.43				IDA
9	64279-09	3	F	3.7	43.4	11.7	22.6	1.4	6.08	65	44.6	424.3	22.53	13.75				IDA
10	67985-09	12	M	10.4	76	27.9	36.8	3.72	28.27	47.1	36	242	381.7	16.07	97	2.5	0.5	CD
11	67991-09	5	M	8.4	80.4	22.3	27.7	2.98	23.96	53.3	22	430.5	21.5	5.59				IDA
12	68124-09	6	F	5.8	56	21.6	21.3	2.15	12.04	45	22	393.8	1.45	5.59				IDA
13	68973-09	5	F	9.7	58.7	24.2	14.2	3.03	17.79	42.5	22	394.8	4.54	6.2				IDA
14	69240-09	5	F	5.5	77.9	26.4	34	2.08	16.20	51.3	22	470.5	0.5	4.59				IDA
15	18455-10	9	F	10.69	39	17.51	27.5	5.79	22.58	37	275	338.9	187.9	81.14	91.4	6	2.6	β-TT
16	18492-10	12	F	4.6	66	24.6	21.5	5.36	35.38	42.1	165.	267.1	307.3	62.04	16.4	2,5	81.1	β-TM
17	18497-10	8	F	5.8	56.4	20.6	30.5	3.21	18.10	42.1	38.3	343.6	97.57	11.15	97.4	2.6		CD
18	23744-10	9	F	10.3	39	17.49	18.07	5.89	22.97	31.07	21	349.37	5.54	6.01	92.1	5.9	2	β-TT
19	23760-10	6	F	4.1	55.9	13	22.3	3.15	17.61	48.8	25.9	401.2	0.5	6.47				IDA
20	23774-10	2	F	4	55.3	12.8	23.1	3.12	17.25	48.6	23	397.2	16.96	5.79				IDA
21	23775-10	11	M	4.3	55.9	13	22.1	2.17	12.13	49.5	22	472.4	0.5	4.66				IDA
22	23791-10	2	F	4.5	55.7	10.8	21.5	2.17	12.09	48.4	21.2	399.6	4.81	5.31				IDA
23	23793-10	3	F	6.2	50.1	13.7	22.1	3.15	15.78	42.1	43	100	1855	71.59	97	2.5	0.5	CD

24	23841-10	1	F	6.2	59.1	19.3	21.1	2.03	12.00	72.5	23	373.2	3.43	6.16				IDA
25	31921-10	11	M	8.1	55.1	18.7	21.1	3.75	20.66	42.1	29.8	359.9	235.1	82.8	97.5	2	1.5	CD
26	31979-10	2	M	4.7	62.1	13.2	22.1	3.73	23.16	41.7	199.	278.3	385.1	71.79	10.5	2.5	87	β-TM
27	33824-10	2	F	10.4	78	21.5	25.5	3.99	31.12	47.5	328	558	6.79	58.78				IDA
28	34721-10	2	M	5.9	60.5	23	28.5	2.57	15.55	67.3	22	427	1.45	5.59				IDA
29	35734-10	10	M	9.3	73.1	20.9	28.5	3.99	29.17	43.1	23	470	0.5	5.72				IDA
30	35941-10	11	M	4.9	65.2	13.2	21.2	2.12	13.82	47.1	21	401.3	16.74	5.31				IDA
31	36791-10	12	M	8.4	55.1	17.7	21.3	3.15	17.36	46.1	20	424.3	15.7	4.8				IDA
32	37921-10	5	M	4.6	65.2	13.2	21.2	2.12	13.82	50.4	23	480.3	0.92	4.71				IDA
33	37979-10	3	F	5.9	61.5	21	23.5	3.17	19.50	53	20	410.3	3.41	5.38				IDA
34	37999-10	5	M	9.7	55.7	13.7	20.2	2.12	11.81	49	20	398.1	12.47	5.59				IDA
35	39731-10	7	M	9.8	63.1	21.7	20.2	3.14	19.81	53	23	399.7	11.47	3.72				IDA
36	39941-10	9	F	10.71	38.73	16.11	25	5.99	23.20	37	97.1	279	237	82.8	12	87.5	0.5	β-TM
37	40020-10	3	F	7.8	58	20.1	23	2.05	11.89	52.3	22	391.7	12.4	7.46				IDA
38	40120-10	5	M	5.4	57	20.1	22.1	2.05	11.69	50.4	22	421.7	1.51	5.52				IDA
39	40320-10	7	M	3.4	61.4	16.5	23.1	2.06	12.65	52.6	23	474.1	0.5	4.73				IDA
40	40341-10	9	F	8.1	70.1	19.1	27.2	3.17	22.22	46	21	434.7	7.4	5.59				IDA
41	41400-10	8	M	6.2	57	19.1	21.3	2.03	11.57	72.5	23	394.7	1.52	5.52				IDA
42	41740-10	11	M	8.4	73	22.3	21.3	3.14	22.92	42.1	22.1	278	235	81.8	96	3.5	1.5	CD
43	42751-10	12	F	8.1	70.1	19.1	27.2	4.25	29.79	46	22	410	9.74	5.59				IDA
44	43761-10	10	M	4.6	65.2	13.2	21.2	3.31	21.58	50.4	21	420	0.75	4.51				IDA

IDA – Iron Deficiency Anaemia

β-TM – Beta Thalassemia Trait

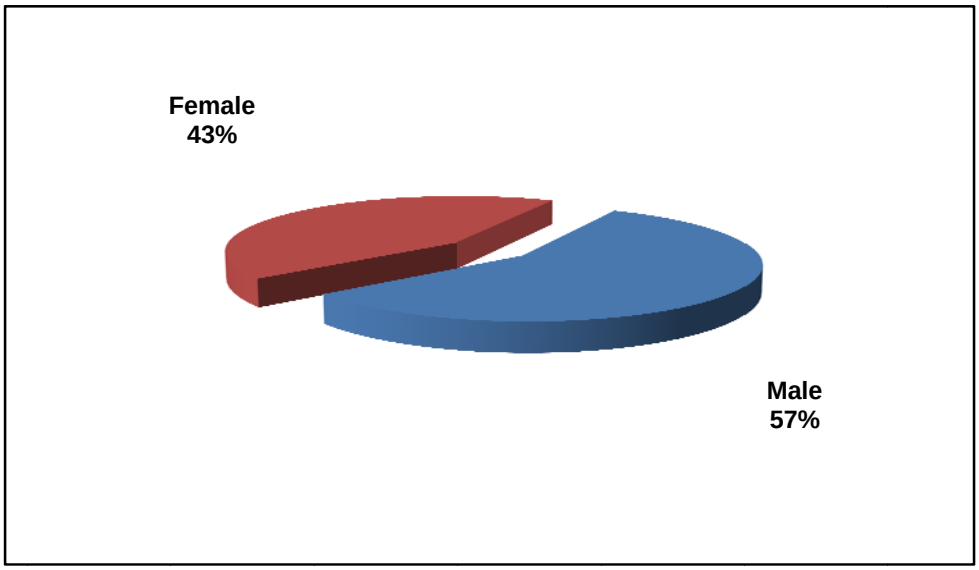
CD – Anaemia of Chronic Disease

ANNEXURE-4

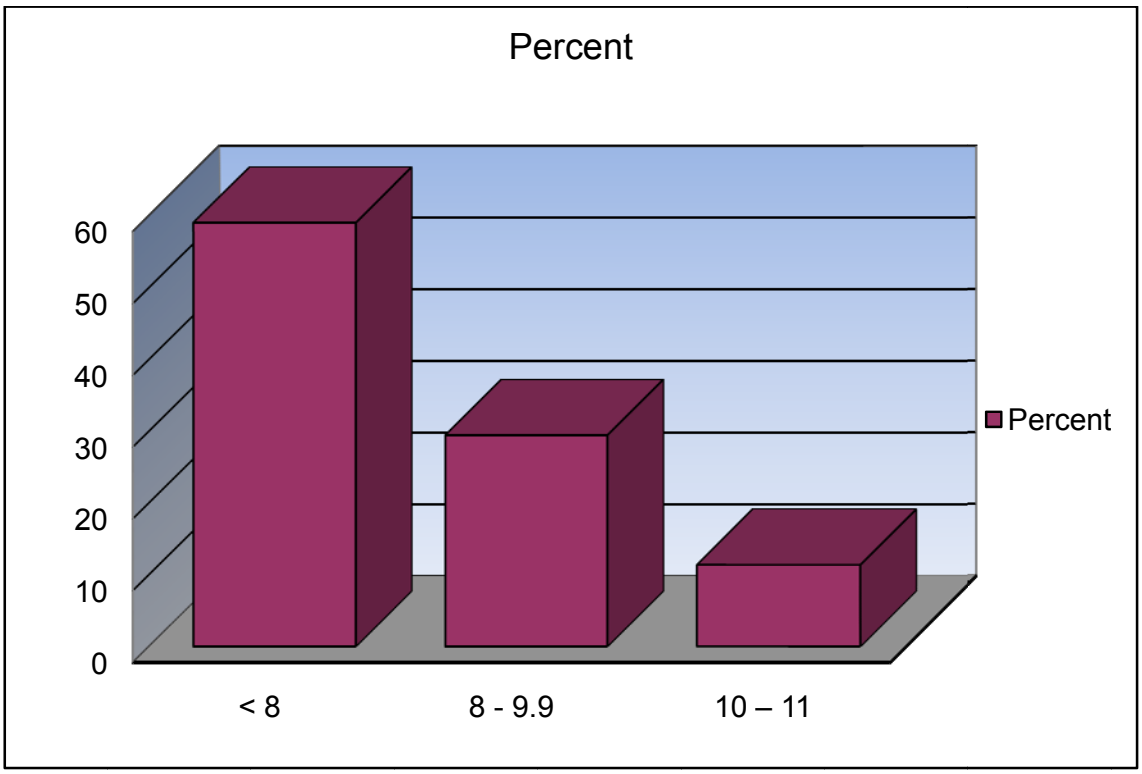
MASTER CHART: 2

Sl. No.	IP No	Mentzer Index	Shine and Lal Index	England Fraser Index	Srivasthava Index	Green & king Index	RBC Distribution width Index	Ricerca Index	Result
1	48837-09	30.66	1544.57	29.91	9.46	374.64	1584.93	19.96	IDA
2	56630-09	18.38	1152.60	20.63	6.37	273.68	869.47	12.55	IDA
3	67991-09	26.98	1441.51	32.02	7.48	410.17	1438.03	17.89	IDA
4	68973-09	19.37	833.86	3.77	7.99	150.97	823.35	14.03	IDA
5	33824-10	19.55	1308.06	18.61	5.39	277.88	928.57	11.90	IDA
6	35734-10	18.32	1116.81	19.21	5.24	247.64	789.63	10.80	IDA
7	36791-10	17.49	537.37	6.55	5.62	166.62	806.38	14.63	IDA
8	37999-10	26.27	425.04	1.68	6.46	156.72	1287.41	23.11	IDA
9	39731-10	20.10	864.01	7.56	6.91	215.33	1065.06	16.88	IDA
10	40341-10	22.11	938.58	23.03	6.03	279.07	1017.22	14.51	IDA
11	42751-10	16.49	938.58	21.95	4.49	279.07	758.73	10.82	IDA
12	18455-10	6.74	266.33	-23.64	3.02	52.64	249.22	6.39	β-TT
13	23744-10	6.62	266.02	-21.79	2.97	45.88	205.73	5.28	β-TT
14	39941-10	6.47	241.65	-24.21	2.69	51.82	239.23	6.18	β-TT

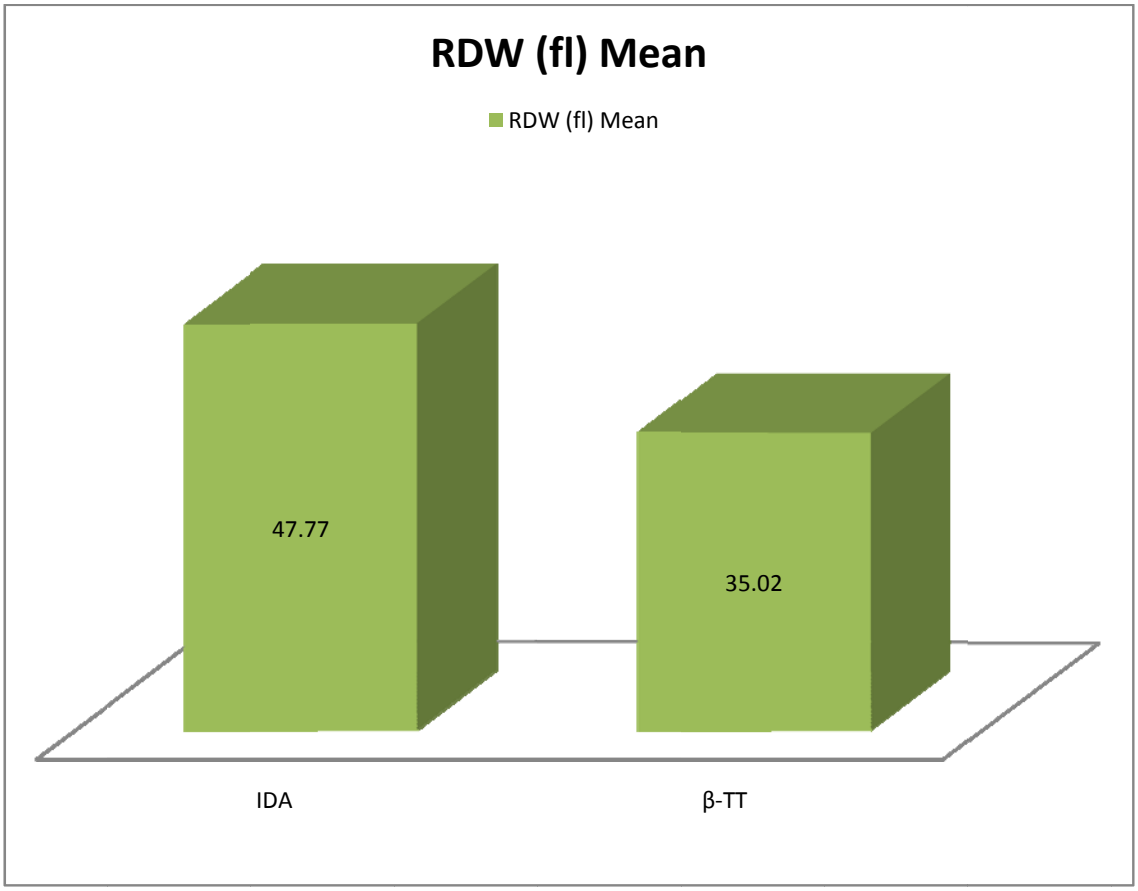
SEX INCIDENCE



SEVERITY OF ANAEMIA



RED CELL DISTRIBUTION WIDTH (fl)



MEAN CORPUSCULAR VOLUME (fl)